REVIEW ARTICLE

Plasma fibrin clot structure and thromboembolism: clinical implications

Michał Ząbczyk, Anetta Undas

Institute of Cardiology, Jagiellonian University School of Medicine
John Paul II Hospital, Kraków, Poland

KEY WORDS

anticoagulation, clot permeability, fibrin, recurrence, thrombosis

ABSTRACT

Fibrin formed as a result of fibrinogen polymerization is the main protein component of a clot in a test tube and intravascular thrombi in vivo. Fibrin clot structure characterized by fiber diameter and pore size differs between healthy persons and those with thromboembolic diseases, in part due to the quality and quantity of fibrinogen and the magnitude of thrombin generation. A key measure of plasma clot structure is its permeability, reflected by the Darcy constant (K_s). Reduced K_s is a typical feature of the prothrombotic fibrin clot phenotype, which is associated with faster formation of denser fibrin mesh, relatively resistant to lysis. Low K_s has been reported in patients with prior or acute myocardial infarction (MI), stroke, or venous thromboembolism (encompassing deep vein thrombosis [DVT] and pulmonary embolism [PE]), as well as in those with prothrombotic conditions (eg, in several thrombophilic states) and in the presence of cardiovascular risk factors (eg, obesity). Antithrombotic and anticoagulant agents, along with statins, have been shown to increase K_s. Growing evidence indicates associations between the properties of plasma fibrin clots and morphology of intravascular thrombi in patients with MI. Recently, reduced K_s has been shown to predict recurrent thromboembolic episodes in patients with a history of stroke, PE, DVT, and their serious complications, including postthrombotic syndrome and thromboembolic pulmonary hypertension. We discuss the current evidence for the significance of clot density measured in vitro as a prognostic marker in a number of clinical conditions associated with elevated thromboembolic risk.

Correspondence to:
Prof. Anetta Undas, MD, PhD, Instytut Kardiologii, Uniwersytet Jagielloński, Collegium Medicum, ul. Prądnicka 80, 31-202 Kraków, Poland, phone: +48 12 614 30 04, email: mmundas@cyf-kr.edu.pl
Received: December 8, 2017.
Accepted: December 8, 2017.
Published online: December 11, 2017.
Conflict of interest: none declared.
Pol Arch Intern Med. 2017; 127 (12): 873-881
doi:10.20452/pamw.4165

Fibrin formation and structure Fibrin is the main protein component of a blood clot and intravascular thrombi in all locations. Efficient fibrin formation and its normal functions are essential for hemostasis.[1] Fibrinogen, the soluble fibrin precursor synthesized in the liver, is a 340-kDa glycoprotein composed of 3 paired polypeptide chains $(A\alpha B\beta\gamma)_2$ that are cross-linked together by 29 disulfide bonds. Fibrinogen contains 3 main structural regions connected by α-helical coils: a central E domain composed of the N-termini of all 6 polypeptide chains and 2 outer D domains with C-termini of the Bβ and γ chains. The C-terminal of the Aα chain is a globular structure located near the central E domain. Approximately 10% of total plasma fibrinogen molecules contain γ' chain, whose presence may contribute to cardiovascular disease.[1-3]

Fibrin formation is initiated by thrombin cleavage of the Aα and Bβ chains of fibrinogen.[3] Thrombin specifically cleaves fibrinopeptides A and—much slower—B from the N-termini of the Aα and Bβ chains, respectively, resulting in the formation of fibrin monomer with exposed binding sites in the E domain. Fibrin monomers polymerize via noncovalent interactions between the D and E domains with subsequent lateral aggregation promoted mainly by intermolecular cross-linking of α chains and probably by interactions between α and γ chains.[3,4] A half-staggered fibrin structure forms a protofibril.

Fibrin resistance to plasmin degradation is determined largely by covalent cross-linking mediated by activated factor XIII (FXIII), a transglutaminase enzyme whose formation from zymogen FXIII is catalyzed by thrombin. Active FXIII catalyzes the formation of covalent bonds between γ-γ, γ-α, and α-α chains of contiguous fibrin polypeptide chains.[5] FXIII also links α_2-antiplasmin and plasminogen activator inhibitors to fibrin to ensure clot resistance to enzymatic degradation.[5] Fibrinogen and fibrin specifically bind a variety

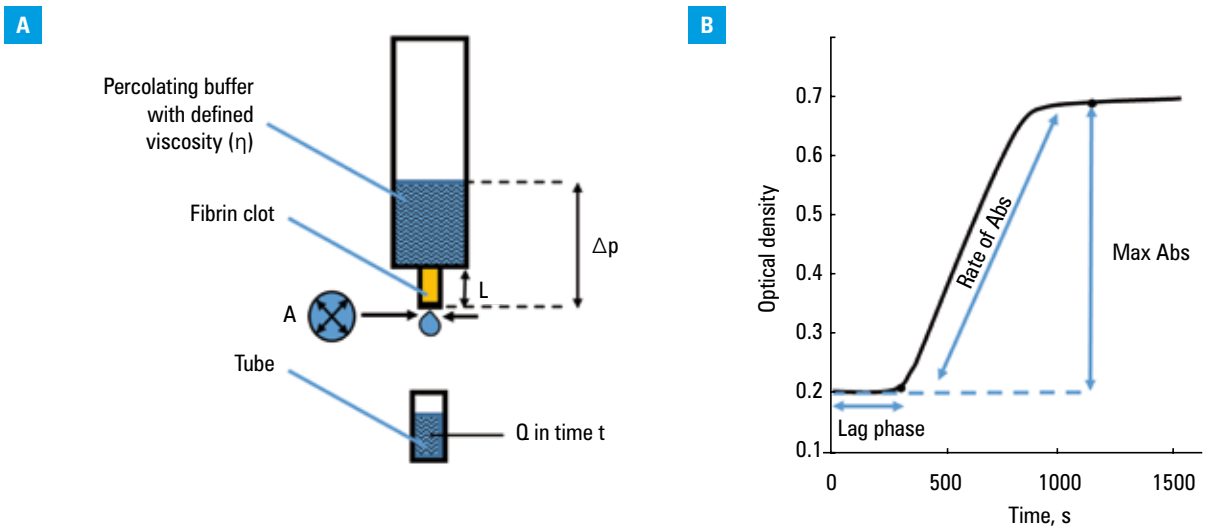

FIGURE 1 **A** – schematic presentation of the system used to assess fibrin clot porosity by measuring its permeability under a hydrostatic pressure using the clot permeability formula; **B** – turbidimetric measurement of fibrin clot formation following addition of calcium and thrombin to plasma
Abbreviations: A, cross-sectional area; Abs, absorbance; L, clot length; Q, volume of the fluid; Δp, differential pressure

of other proteins, including albumin, apolipoproteins, complement C3, ferritin, fibronectin, fibulin, fibroblast growth factor 2, haptoglobin, interleukin 1β, myosin, plasminogen, thrombospondin, histidine-rich glycoprotein, vitronectin, vascular endothelial growth factor, and von Willebrand factor.[6]

Mechanical properties of fibrin The structure of fibrin and its resistance to mechanical deformation and/or enzymatic degradation are essential for fibrin functions and depend on fibers' architecture and their individual properties.[1,3] At the level of the individual fibrin monomers, the coiled-coil connectors, folded globular nodules, and αC regions largely contribute to the fibrin clot mechanics.[7] Fibrin networks have 1.5-to 3-fold lower extensibilities than individual fibers, thus clot degradation after extension is limited to the branch points rather than single fibers.[8] Fibrin fibers are composed of thousands of twisted protofibrils arranged side by side with a periodicity of 22.5 nm.[9] Extremely durable cross-linked fibrin fibers can be strained over 6 times their length and they have the largest extensibilities among fiber proteins.[8]

Assessment of fibrin properties The architecture of a fibrin clot can be characterized by the fiber diameter and the size of the pores in the fibrin network. Fibrin porosity is typically estimated using a clot permeability under different hydrostatic pressures in a few assays of varying reagents and their concentrations.[10] Clot permeability is calculated based on the volume of a buffer flowing through a fibrin gel in a given time period using the Darcy constant: K_s ($\times 10^{-9}$ cm^2) = $Q \times L \times \eta / t \times A \times \Delta p$, where Q is the volume (in ml) of liquid passed through in time t, η is the viscosity of the fluid (in poise), L is the length of the gel (in cm), A is a cross-sectional area (in cm^2), and p is the applied pressure (in dyne/cm^2) (FIGURE 1A).

Clot compaction is a measure of fibrin density, thus indirectly clot permeability. After clot formation, samples are centrifuged at 6000 to 8000 × *g* for usually 60 seconds and the volume of the supernatant evacuated from tubes is assessed as a difference in tube weight. Finally, clot compaction is expressed as a volume of the supernatant divided by the initial plasma volume used to form the clot. A normal clot compaction is about 60% to 70% using this technique. Less compact clots (usually about 40%) are associated with impaired clot lysis due to impaired transport of proteolytic enzymes through the fibrin network.

Fibrin formation in purified systems as well as in plasma are assessed by turbidimetry. The parameters used are the lag phase that reflects the time to the start of lateral fibril aggregation, the maximum absorbance of the growing clot that reflects an average fibrin fiber size and the number of protofibrils per fiber, and the rate of absorbance increase (FIGURE 1B).[10,11]

The most commonly used imaging techniques for visualization of fibrin clot structure include scanning or transmission electron microscopy, along with confocal microscopy.[10] Scanning electron microscopy allows a measurement of the fiber diameter, pore size, and branching angles. However, this technique requires a clot fixation with glutaraldehyde solution followed by its dehydration. Physiologically, fibrinogen undergoes many posttranslational modifications, thus purified models compared with plasma-based models can mimic the in vivo situation. Fibrin clot formed from a citrated plasma is characterized by 2 to 4 times thicker fibers and larger pores compared with a clot formed from purified fibrinogen at similar protein levels.[12] Fibrinogen is known as the main determinant of fibrin clot structure; however, variation in its levels explained only 18% of the variation in K_s and 48% of the variation in maximum absorbance in turbidimetry.[13]

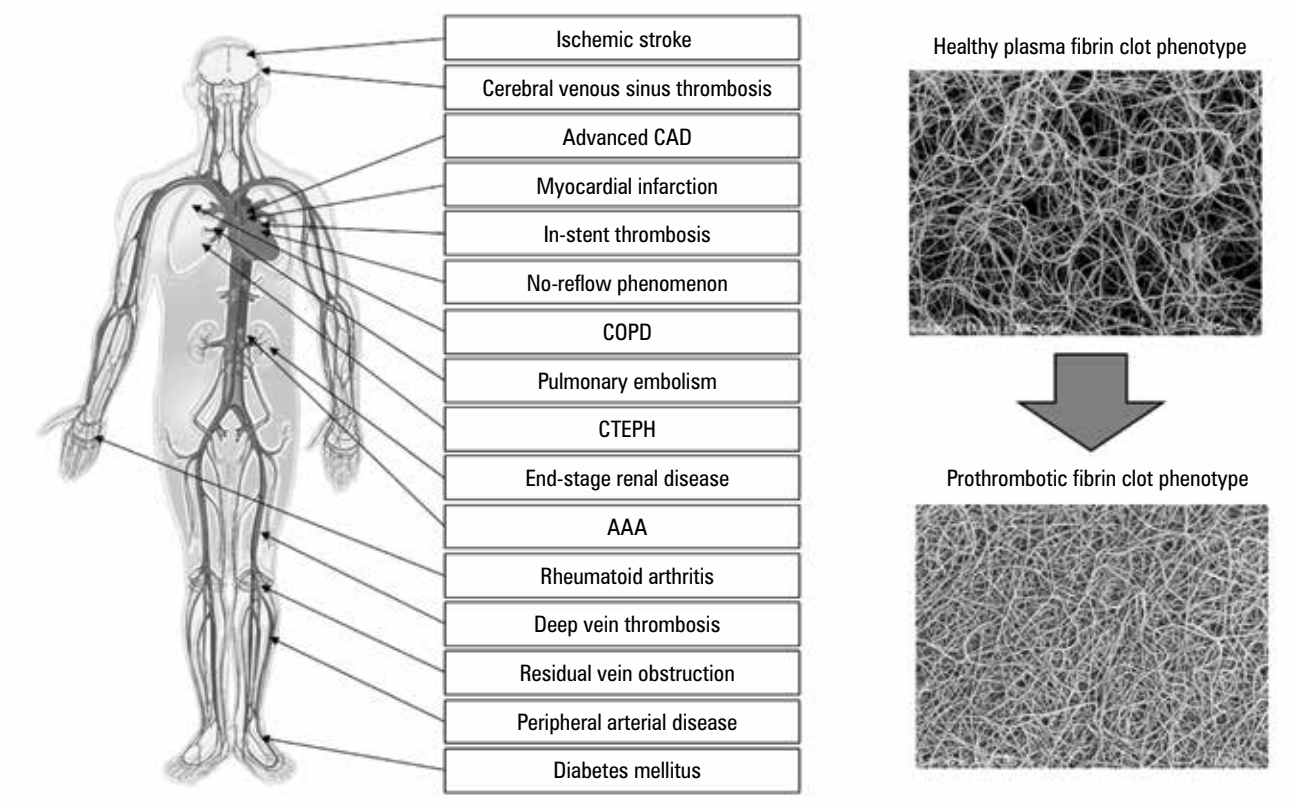

FIGURE 2 Clinical conditions in which prothrombotic fibrin clot phenotype has been reported
Abbreviations: AAA, abdominal aortic aneurysm; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CTEPH, chronic thromboembolic pulmonary hypertension

At low concentrations of fibrinogen, clots are composed of thicker fibers, while clots formed at higher concentrations of fibrinogen are composed of thinner fibers. Clot structure also depends on the conditions during fibrinogen polymerization.[2] Increased thrombin concentrations in a dose-dependent manner result in faster formation of thinner fibrin fibers with smaller pores.[14] Thus, higher thrombin generation is directly associated with lower clot permeability. Elevated FXIII activity has also been shown to affect clot structure by increasing its stability.[15] Generally, fibrin clots are composed of thinner fibers and smaller pores and are more compact and less permeable, while those with thicker fibers are more permeable and susceptible for fibrinolysis.[16,17]

In 1977, Carr et al[18] estimated for the first time the clot permeability by measuring the buffer flow through a fibrin gel. Currently, several methods and models are used to determine fibrin porosity, including those in which clotting is initiated by thrombin or tissue factor in platelet-poor or platelet-rich plasma, using manual or semiautomated techniques.[19-23] In 2012, the Factor XIII and Fibrinogen Subcommittee of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis published the first recommended protocol for K_s measurement.[24] Regardless of the assay used, K_s measurement requires much hands-on experience to obtain reproducible results (variability around 10% or less) and universal standards to ensure acceptable between-laboratory variability.[20]

This review will focus on reduced fibrin clot permeability, which represents the so-called prothrombotic fibrin clot phenotype in patients with thromboembolic disorders, in particular on myocardial infarction (MI), stroke, and venous thromboembolism (VTE), including pulmonary embolism (PE) and deep vein thrombosis (DVT). Clinical conditions associated with reduced fibrin clot permeability are shown in FIGURE 2.

Fibrin clot phenotype in myocardial infarction

Acute coronary artery thrombus formation associated mostly with atherosclerotic plaque rupture results in a blood flow cessation in an infarct-related artery area, leading to ST-segment elevation MI (STEMI). Scanning electron microscopy of intracoronary thrombi from patients with STEMI showed that thrombus composition evolves over time during the acute phase of MI and fibrin content increased from 31% to 78%.[25] Fibrin, erythrocyte, platelet, and white blood cell content in the thrombi were estimated at 49.1%, 24.2%, 11.6%, and 3.7%, respectively.[25] Interestingly, higher content of fibrin (61.6% vs 34.3%) and platelets (8.2% vs 4.8%) and lower erythrocyte content (15.8% vs 42.9%) were found on the intracoronary thrombus surface compared with its inner part.[26] In patients with acute MI before percutaneous coronary intervention (PCI), fibrinogen, P-selectin, and plasminogen activator

inhibitor 1 (PAI-1) were positively correlated with thrombus fibrin content.[25] Diminished clot permeability in 40 patients with acute MI was related to the degree of oxidative stress and inflammation.[27] Different oxidative modifications of fibrinogen molecule were associated with various changes in the clot structure and function.[28-31] Serum F2-isoprostanes produced upon nonenzymatic arachidonic acid peroxidation, as well as platelet activation reflected by the concentrations of β-thromboglobulin, have been shown to correlate with K_s in patients with acute MI.[29]

Zalewski et al[26] showed for the first time that fibrin content in the intracoronary thrombi obtained from patients with acute MI was inversely correlated with K_s, and the latter variable independently predicted fibrin content in the whole thrombus. This indicates that plasma clot features affect thrombi formed in the vessels, which supports the value of plasma clot analysis in the prediction of in vivo thrombus composition.

The presence and amount of the proteins within a plasma clot also influence clot properties.[11] Using a shotgun proteomic method to investigate time-dependent protein composition of clots prepared ex vivo from citrated plasma obtained from patients with acute MI, 62 proteins were identified in all 8 samples grouping into several distinct functional clusters (eg, cholesterol transporter activity, immunoglobulin binding, and peptidase regulatory activity).[32] The protein signatures of clots differed significantly depending on time after acute coronary syndrome, showing 30% greater variability in protein composition of the plasma clots generated 2 months after the onset of acute MI.[32] Differences involving proteins of potential influence on within-clot fibrinolysis (ie, α_2-antiplasmin) may at least in part explain time-dependent changes in the clot structure in patients with acute MI.[32] Before PCI, fibrinogen, P-selectin, and PAI-1 levels were positively correlated with thrombus fibrin content, while after PCI, von Willebrand factor antigen, soluble CD40 ligand, and myeloperoxidase were associated with thrombus fibrin content.[25] After adjustment for fibrinogen and the onset-to-thrombectomy time, circulating von Willebrand factor antigen was the independent predictor of fibrin-rich intracoronary thrombi.[25]

Reduced clot permeability by about 30% has been reported for the first time in men with a history of premature MI.[33] Thus, thrombotic disorders can be attributed to more compact and less permeable fibrin structure.[34] MI in the past has also been linked with the prothrombotic fibrin clot phenotype in young patients.[35] Interestingly, first-degree male relatives of patients with premature coronary artery disease had less permeable clots than controls.[19] Reduced clot permeability by 21% and 18%, respectively, characterized also patients with in-stent thrombosis[36] and patients with a history of the no-reflow phenomenon,[37] defined as the absence of a complete myocardial perfusion despite successful opening of the blocked coronary artery. A small prospective study demonstrated an increased risk of arterial thrombotic events associated with reduced K_s and prolonged lysis time in patients on long-term hemodialysis.[38]

Altered fibrin clot structure and function observed in MI is largely determined by factors associated with increased oxidative stress and thrombin generation, along with prothrombotic actions of platelet-derived proteins (eg, platelet factor 4 or β-thromboglobulin).[11,26,28,39] Hyperglycemia, observed in up to 50% of the patients with acute MI, impaired clot susceptibility to lysis but had no effect on K_s.[40] On the other hand, patients with type 2 diabetes presented impaired clot structure, including its reduced K_s, associated with prolonged duration of diabetes.[41] This observation underlines the role of prothrombotic memory in the pathogenesis and risk assessment of thrombotic events.[41] Interestingly, cellular fibronectin, a marker of vascular injury, was increased and associated with altered fibrin clot properties in patients with type 2 diabetes and concomitant cardiovascular disease but not in patients without cardiovascular disease, suggesting a direct link between blood vessel damage and prothrombotic clot phenotype.[42]

Increased oxidative stress, platelet activation, and thrombin generation play a key role in the pathogenesis of MI. Growing evidence indicates that clots formed in the presence of oxidants and high thrombin concentrations are compact, composed of thin fibers, and have reduced K_s.[30,43,44] Thus, fibrin clot porosity is a promising prognostic marker in patients with MI.

Fibrin clot phenotype in stroke Precerebral or cerebral artery occlusions constitute a major percentage of all acute strokes. Thrombi retrieved from the cerebral arteries of patients with acute ischemic stroke contained fibrin and platelet deposits, together with erythrocyte components.[45] Fibrin content in thrombi obtained from 50 patients with acute middle cerebral artery ischemic stroke was 61%, and fibrin-rich thrombus occurred most commonly among these patients.[46] Acute ischemic stroke within the first 72 hours of symptom onset was associated with K_s reduced by 30% as compared with healthy controls.[47]

Fibrin properties showed an association with stroke severity but not with mortality during long-term follow-up.[48] Fibrin clot compaction correlated with neurological deficit both on admission and at discharge of patients admitted for acute ischemic stroke.[47] Formation of denser fibrin clots displaying impaired lysability and pattern of their changes induced by thrombolysis may affect clinical outcome in patients with acute ischemic stroke.[49] We have reported recently that in stroke patients eligible for recombinant tissue plasminogen activator (rtPA) treatment, fibrin networks assessed 24 hours since thrombolysis were 37% less compact and lysed 21% more rapidly compared with the pretreatment

values.[49] Moreover, thrombolysis with rtPA within 4.5 hours since stroke onset markedly attenuated thrombin generation, including reduced maximal thrombin concentrations, measured at 24 hours with a large contribution of activated factor XI which was found in about 15% of patients.[50]

Patients who survived ischemic stroke have also been characterized by abnormal plasma fibrin clot properties. The first report showing prothrombotic clot abnormalities was published for patients with cryptogenic stroke and evidenced more compact plasma clots generated in vitro with an increased fiber diameter and density.[51] The prothrombotic fibrin clot phenotype, including K_s reduced by 7.5% and lysis time prolonged by 13.8%, characterized patients with prior ischemic stroke.[52]

Reduced K_s observed within the first 24 hours since the onset of stroke symptoms in nonthrombolysed patients remained unaltered after 60 days from the event, suggesting that the prothrombotic clot phenotype is a persistent feature of patients suffering from ischemic stroke.[53]

The worse prognosis is observed in patients with atrial fibrillation (AF) who experienced acute cerebrovascular episode. Since 15% of ischemic strokes occur in patients with AF and they are mostly caused by thrombotic material formed within the left atrium, precisely in 90% in its appendage, the prothrombotic fibrin clot phenotype might also increase the risk of cerebral infarction. It has been shown that formation of compact plasma fibrin clots resistant to lysis is observed in patients with different types of AF,[54] and those unfavorable fibrin properties can at least in part determine the efficacy and safety of anticoagulation with vitamin K antagonists.[55] Interestingly, patients with lower K_s ($<6.8\times10^{-9}$ cm^2) had increased risk of ischemic stroke or transient ischemic attack (hazard ratio [HR], 6.55; 95% confidence interval [CI], 2.17–19.82) and major bleeds (HR, 10.65; 95% CI, 3.52–32.22), while patients with elevated K_s ($\geq6.8\times10^{-9}$ cm^2) had an increased rate of minor bleeding compared with the remainder (11.63% per year vs 3.55% per year).[55] Interestingly, in 160 patients with AF, prothrombotic fibrin clot properties were determined by PAI-1 and N-terminal pro-B-type natriuretic peptide associated with long-term prognosis.[52]

Prothrombotic blood alterations could be also involved in the left atrial appendage thrombus formation in patients without documented AF and are associated with increased risk of stroke or transient ischemic attack.[56] K_s reduced by 1 unit was shown to be associated with 5-fold higher odds of recurrent left atrial appendage thrombus during follow-up, which suggests that clot features persist over time and might have a prognostic value.[56] Fibrin clot features could be involved in an increased stroke risk also in specific patient populations, including those with acquired thrombophilia. We found that plasma fibrin clots from patients with antiphospholipid syndrome, who experienced stroke and/or MI at least 5 months earlier, were less permeable and were lysed faster compared with those with VTE alone.[57] This study showed for the first time that antiphospholipid syndrome is associated with the prothrombotic plasma fibrin clot phenotype, with even worse characteristics in patients following arterial thrombosis, largely stroke.[57]

Overall, ischemic stroke is linked with prothrombotic alterations in fibrin structure and function, indicating common mechanisms leading to cerebrovascular and coronary thromboembolic episodes.[11,34]

Fibrin clot phenotype in venous thromboembolism The pathogenesis of VTE, encompassing DVT and PE, is multifactorial with about 50% of patients suffering from unprovoked (formerly idiopathic) episodes. Thrombotic material removed from the pulmonary arteries in acute PE showed that the main component of the thrombus is fibrin.[58,59] Interestingly, thrombi located in the distal arteries were richer in fibrin and contained fewer erythrocytes than those from the proximal arteries.[58] Martinez et al[60] showed that clot structure and functional properties, including clot formation, lysis, and viscoelastic properties, differentiated patients with acute DVT from those with PE, suggesting that lower fiber density and faster lysis in patients with PE may affect embolization. Less compact fibrin structure associated with faster clot lysis seems to characterize patients who experienced PE, regardless of the presence of concomitant DVT.[61] It might be speculated that higher K_s combined with shorter lysis time, found in patients with PE compared with DVT, contributes to clot fragmentation and the subsequent embolization.[61] Recently published computational model simulations describing interactions between main clot components under shear flow suggest that blood clots with higher clot shell permeability are more prone to embolization under increasing shear rate.[62]

It has been reported that some environmental factors affect fibrin clot structure in patients with DVT. Long-term exposure to particulate matter with a diameter of less than 10 μm (PM_{10}) has been associated with a denser fibrin network with K_s decreased by 22% in 103 patients with DVT but not in controls, suggesting an increased risk of recurrent episodes in patients predisposed to venous thrombosis.[63]

Siudut et al[64] showed that also cerebral venous sinus thrombosis (CVST), which is a rare form of VTE, can be associated with the formation of more compact plasma fibrin clots and resistance to fibrinolysis. K_s was 12% lower in 50 patients with CVST (mean [SD] age, 38.9 [9.8] years) after the event unrelated to trauma or malignancy following anticoagulation withdrawal, as compared with controls.[64] Importantly, recurrent CVST during follow-up was associated with 21% higher baseline fibrinogen levels and 20% lower K_s,[64] which suggest a predictive value of this fibrin clot structure measure (FIGURE 3).

FIGURE 3 Relative differences in plasma fibrin clot permeability (ΔK_s) measured after 3 to 6 months since the index event between patients who experienced recurrent venous thromboembolic (VTE) episodes, including cerebral venous sinus thrombosis (CVST), deep vein thrombosis (DVT), and pulmonary embolism (PE) during a few years' follow-up versus those free of such events in the respective studies.[64,65,67] K_s for patients without recurrent VTE is marked on the top of the vertical line.

Recent data indeed indicate that plasma clot characteristics might identify patients at high risk of recurrent VTE. In 2017, we observed that recurrent PE, diagnosed during follow-up in 23 patients (5% per year) following the first-ever provoked or unprovoked PE, is associated with formation of denser fibrin networks, as evidenced by reduced K_s (HR, 1.82; 95% CI, 1.01–3.13; FIGURE 3).[65]

Postthrombotic syndrome is a common complication of DVT that has been found to be associated with K_s reduced by 11.7% when assessed 3 months after the index DVT.[66] Recurrent VTE occurred also more commonly in patients with postthrombotic syndrome, who were characterized by lower K_s and prolonged clot lysis time (CLT).[66]

Very recently, we have published convincing evidence for the predictive value of the prothrombotic clot phenotype in patients with DVT. Among 320 consecutive patients after the first-ever DVT, recurrences were observed in 25% of the patients (6.6% per year) during a median follow-up of 44 months. Recurrent DVT was associated with denser fibrin networks (12% lower K_s), and reduced K_s was an independent predictor of recurrences.[67] The risk of recurrent DVT increased 15-fold (95% CI, 7.5–33.5) in patients with low K_s ($\leq 7.3 \times 10^{-9}$ cm^2) combined with prolonged CLT (>96 min).[67] Interestingly, fibrin properties analyzed as single measures were less effective as DVT predictors when compared with the combined analysis of K_s and CLT (FIGURE 3).[67]

This work encourages efforts to develop user-friendly assays to test clot properties in practice to improve identification of patients with VTE at risk of recurrent episodes following anticoagulation cessation. The prothrombotic clot phenotype could be a factor which renders the patient suitable for long-term anticoagulation, now easier to achieve with new oral anticoagulants available worldwide.

Patients with residual vein obstruction, detected in up to 50% of patients following DVT, were also characterized by 14% lower K_s related to elevated lipoprotein(a) and overrepresentation of its smaller isoforms.[68]

Chronic thromboembolic pulmonary hypertension (CTEPH), a severe PE complication, is associated with changes in fibrinogen molecule structure and/or function followed by altered properties of the clot formed from modified protein.[69] This might result in the development of CTEPH by prolonged fibrin clot presence within the pulmonary arteries and remodeling of the thrombi into fibrotic layer. Patients with CTEPH have a relatively high incidence of inherited dysfibrinogenemias in which abnormal fibrin clot phenotype occurs,[70,71] suggesting that clot properties may lead to identification of patients at risk of this complication and might be helpful in determining the efficacy of therapy.[72]

Looking at genetic thrombophilias attributed to VTE, fibrin abnormalities have also been reported. The prothrombin G20210A mutation in patients after VTE has been shown to be linked with unfavorable fibrin clot characteristics, including 12% lower K_s, compared with noncarriers.[73] Rivaroxaban treatment improved fibrin clot properties in carriers and noncarriers of the prothrombin G20210A mutation, but anticoagulant therapy cannot abolish more prothrombotic fibrin clot phenotype observed in prothrombin mutation carriers following VTE.[73] Factor V (FV) Leiden mutation is observed in 3% to 15% of the white population and in 15% to 30% of patients with VTE.[74] In thrombosis-free heterozygous carriers of FV Leiden mutation, K_s was slightly reduced compared with noncarriers.[74]

TABLE 1 Medications reported to alter fibrin clot permeability

Medication	Mode of action: suggested change in
Metformin	Glycation of fibrinogen and plasminogen; inflammatory state
Insulin	Glycation of fibrinogen and plasminogen; inflammatory state
Statins (simvastatin and atorvastatin)	Thrombin generation; inflammatory state
Aspirin	Acetylation of fibrinogen
Angiotensin converting enzyme inhibitors	Fibrinolysis
Vitamin K antagonists (warfarin, acenocoumarol)	Thrombin generation via vitamin K-dependent factors
Non–vitamin K antagonist oral anticoagulants (rivaroxaban, apixaban, dabigatran)	Thrombin generation by activated factor X or thrombin inhibition
Heparins	Thrombin generation by activated factor X inhibition

Among modifiable VTE risk factors, oral contraceptives have been shown to unfavorably alter plasma clot characteristics.[74] Discontinuation of oral contraceptives was associated with shortened lysis time and increased K_s.[74]

It is known that obesity is associated with an increased risk of thromboembolic events. Interestingly, in 29 obese patients studied before and after 3-month low-fat diet, which resulted in the reduction of body weight and the levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, and PAI-1, was also associated with increased clot potential to lysis, while K_s remained unchanged.[75]

Taken together, there is evidence that a number of thrombosis risk factors, both genetically determined and acquired, are associated with denser plasma clot structure, which might contribute to their effects reaching beyond the impact of increased thrombin generation.

Antithrombotic agents and fibrin clots Growing evidence indicates a strong impact of several antithrombotic, cholesterol-lowering, or antihypertensive agents on fibrin clot structure (TABLE 1). Administration of low-dose aspirin was shown to be associated with improved clot properties largely through fibrinogen acetylation.[76,77] Fibrinogen is acetylated at several lysine residues, which also are involved in the FXIII-mediated cross linking of fibrin.[77] In vivo, aspirin concentration of 37.5, 320, and 640 mg/d increased K_s by 44%, 31%, and 21%, respectively, and treatment with low-dose aspirin led to formation of thicker fibrin fibers with larger pores.[78] Similar effects of low-dose aspirin were seen in patients with coronary artery disease and in those with VTE.[79] Moreover, 1 week after aspirin withdrawal, K_s returned to the baseline value.[79]

The impact of several other antithrombotic drugs on fibrin clot structure and function was determined by the influence on clotting factors and/or reduced thrombin generation.[80,81] Direct and indirect thrombin inhibitors, including argatroban, bivalirudin, and danaparoid, have been shown to increase K_s at therapeutic concentrations in in vitro models to a similar extent.[82]

Direct thrombin and factor Xa inhibitors added in vitro to normal plasma also increased K_s by 36% to 91% at therapeutic plasma concentration.[83] In the same model, warfarin at plasma concentration adjusted to obtain the therapeutic range (international normalized ratio, 2–3) resulted in an K_s increase of about 35%. Warfarin and acenocoumarol as vitamin K antagonists have been shown to increase K_s in patients with AF as early as after 3 days of treatment, reaching the plateau value after 7 days.[81] Those favorable changes in clot structure were strictly associated with diminished activities of vitamin K-dependent clotting factors and lower protein C activity, resulting in lower thrombin generation. Rivaroxaban, a non–vitamin K antagonist oral anticoagulant and direct Xa inhibitor, at 2 to 6 hours after intake improved K_s by 37% in patients with previous VTE.[73] Interestingly, after 20 to 25 hours since rivaroxaban intake, clot permeability returned to the baseline value.[73]

Statins also produce fibrin modulatory effects. Atorvastatin, beside its role as a cholesterol-lowering agent, has also antithrombotic properties in VTE patients, observed as favorable alterations in fibrin clot phenotype, in particular K_s reduced by 23% after statin use for 3 days.[84] Furthermore, anticoagulant effects of statins have been related to a decreased tissue factor expression followed by reduced thrombin generation, attenuation of procoagulant reactions catalyzed by thrombin, such as fibrinogen cleavage, FV and FXIII activation, and enhanced endothelial thrombomodulin expression, resulting in increased protein C activation and inactivation of activated FV.[85] A 3-month simvastatin treatment increased K_s in patients without history of cardiovascular events and low density lipoprotein cholesterol of less than 3.4 mmol/l.[86] Beneficial effects of simvastatin treatment on K_s (an increase of 16%), irrespective of cholesterol reduction, were also seen in patients with chronic obstructive pulmonary disease.[87] These findings provide evidence that fibrin clot modulation contributes to antithrombotic effects of statins, which explains at least in part a reduced VTE risk in statin users observed in several studies.

Practical implications There are several limitations to assess fibrin clot properties in clinical practice. The availability of fibrin measures is limited to a few specialized laboratories. Due to

high between-laboratory variability of the methods describing fibrin clot structure and function, the standardization of assays is highly required. A well-trained technical staff with great hands-on experience can support this process; however, still an automatization of manual methods is needed to reduce operator-related bias.[23] Recently, we have developed a semiautomated device controlled by software to assess K_s quickly and precisely after clot washing.[23] Even though the clot preparation has to be conducted manually, our device allows for an analysis of additional parameters describing clot structure and kinetics of buffer permeation through the fibrin mesh.[23] Such devices are needed to implement K_s measurements in clinical practice.

Concluding remarks Fibrin clot structure and function is involved in the pathogenesis and prognosis of thromboembolic events. Fibrin clots composed of compact, highly branched networks with thin fibers are resistant to lysis, and such properties have been reported in patients with several diseases with increased risk of thromboembolism. Recent data indicate that the prothrombotic fibrin phenotype assessed in plasma samples might be used as a prognostic marker in VTE and ischemic stroke related to AF. Further research is needed to better understand the role of fibrin properties in thromboembolism and implement these measures in clinical practice.

Funding This work was supported by a grant from the Polish National Science Centre (no., UMO-2015/19/B/NZ5/02 215; to AU).

REFERENCES

1 Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost. 2005; 3: 1894-1904.

2 Farrell DH. Pathophysiologic roles of the fibrinogen gamma chain. Curr Opin Hematol. 2004; 11: 151-155.

3 Lord ST. Molecular mechanisms affecting fibrin structure and stability. Arterioscler Thromb Vasc Biol. 2011; 31: 494-499.

4 Weisel JW, Litvinov RI. Mechanisms of fibrin polymerisation and clinical implications. Blood. 2013; 121: 1712-1719.

5 Muszbek L, Bereczky Z, Bagoly Z, et al. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. Physiol Rev. 2011; 91: 931-972.

6 Weisel JW, Litvinov RI. Fibrin formation, structure and properties. In: Parry DAD, Squire JM. Fibrous proteins: structures and mechanisms. Cham, Switzerland: Springer; 2017: 405-432.

7 Falvo MR, Gorkun OV, Lord ST. The molecular origins of the mechanical properties of fibrin. Biophys Chem. 2010; 152: 15-20.

8 Liu W, Jawerth LM, Sparks EA, et al. Fibrin fibres have extraordinary extensibility and elasticity. Science. 2006; 313: 634.

9 Weisel JW. The mechanical properties of fibrin for basic scientists and clinicians. Biophys Chem. 2004; 112: 267-276.

10 Undas A. How to Assess Fibrinogen Levels and Fibrin Clot Properties in Clinical Practice? Semin Thromb Hemost. 2016; 42: 381-388.

11 Undas A, Ariëns RA. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol. 2011; 31: e88-e99.

12 Carr ME. Fibrin formed in plasma is composed of fibers more massive than those formed from purified fibrinogen. Thromb Haemost. 1988; 59: 535-539.

13 Dunn EJ, Ariëns RA, de Lange M, et al. Genetics of fibrin clot structure: a twin study. Blood. 2004; 103: 1735-1740.

14 Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev. 2007; 21: 131-142.

15 Hethershaw EL, Cilia La Corte AL, Duval C, et al. The effect of blood coagulation factor XIII on fibrin clot structure and fibrinolysis. J Thromb Haemost. 2014; 12: 197-205.

16 Blombäck B, Carlsson K, Fatah K, et al. Fibrin in human plasma: gel architectures governed by rate and nature of fibrinogen activation. Thromb Res. 1994; 75: 521-538.

17 Blombäck B, Okada M. Fibrin gel structure and clotting time. Thromb Res. 1982; 25: 51-70.

18 Carr ME Jr, Shen LL, Hermans J. Mass-length ratio of fibrin fibers from gel permeation and light scattering. Biopolymers. 1977; 16: 1-15.

19 Mills JD, Ariëns RA, Mansfield MW, et al. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. Circulation. 2002; 106: 1938-1942.

20 Sjøland JA. A newoptimizedmethod for the determination of fibrin clot permeability. Blood Coagul Fibrinolysis. 2005; 16: 579-583.

21 Collet JP, Montalescot G, Lesty C, et al. Disaggregation of in vitro preformed platelet-rich clots by abciximab increases fibrin exposure and promotes fibrinolysis. Arterioscler Thromb Vasc Biol. 2001; 21: 142-148.

22 Undas A, Brozek J, Jankowski M, et al. Plasma homocysteine affects fibrin clot permeability and resistance to lysis in human subjects. Arterioscler Thromb Vasc Biol. 2006; 26: 1397-1404.

23 Ząbczyk M, Piłat A, Awsiuk M, et al. An automated method for fibrin clot permeability assessment. Blood Coagul Fibrinolysis. 2015; 26: 104-109.

24 Pieters M, Undas A, Marchi R, et al.; Factor XIII And Fibrinogen Subcommittee Of The Scientific Standardisation Committee Of The International Society For Thrombosis And Haemostasis. An international study on the standardization of fibrin clot permeability measurement: methodological considerations and implications for healthy control values. J Thromb Haemost. 2012; 10: 2179-2181.

25 Sadowski M, Ząbczyk M, Undas A. Coronary thrombus composition: links with inflammation, platelet and endothelial markers. Atherosclerosis. 2014; 237: 555-561.

26 Zalewski J, Bogaert J, Sadowski M, et al. Plasma fibrin clot phenotype independently affects intracoronary thrombus ultrastructure in patients with acute myocardial infarction. Thromb Haemost. 2015; 113: 1258-1269.

27 Undas A, Szuldrzynski K, Stepien E, et al. Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: effects of inflammation and oxidative stress. Atherosclerosis. 2008; 196: 551-558.

28 Hoffman M. Alterations of fibrinogen structure in human disease. Cardiovasc Hematol Agents Medicinal Chem. 2008; 6: 161-180.

29 Martinez M, Weisel JW, Ischiropoulos H. Functional impact of oxidative posttranslational modifications on fibrinogen and fibrin clots. Free Radic Biol Med. 2013; 65: 411-418.

30 Weigandt KM, White N, Chung D, et al. Fibrin clot structure and mechanics associated with specific oxidation of methionine residues in fibrinogen. Biophys J. 2012; 103: 2399-2407.

31 Upchurch GR Jr, Ramdev N, Walsh MT, et al. Prothrombotic consequences of the oxidation of fibrinogen and their inhibition by aspirin. J Thromb Thrombolysis. 1998; 5: 9-14.

32 Suski M, Siudut J, Ząbczyk M, et al. Shotgun analysis of plasma fibrin clot-bound proteins in patients with acute myocardial infarction. Thromb Res. 2015; 135: 754-759.

33 Fatah K, Hamsten A, Blomback B, et al. Fibrin gel network characteristics and coronary heart disease: relations to plasma fibrinogen concentration, acute phase protein, serum lipoproteins, and coronary atherosclerosis. Thromb Haemost. 1992; 68: 130-135.

34 Undas A. Fibrin clot properties and their modulation in thrombotic disorders. Thromb Haemost. 2014; 112: 32-42.

35 Collet JP, Allali Y, Lesty C, et al. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. Arterioscler Thromb Vasc Biol. 2006; 26: 2567-2573.

36 Undas A, Zalewski J, Krochin M, et al. Altered plasma fibrin clot properties are associated with in-stent thrombosis. Arterioscler Thromb Vasc Biol. 2010; 30: 278-282.

37 Zalewski J, Undas A, Godlewski J, et al. No-reflow phenomenon after acute myocardial infarction is associated with reduced clot permeability and susceptibility to lysis. Arterioscler Thromb Vasc Biol. 2007; 27: 2258-2265.

38 Undas A, Kolarz M, Kopec G, et al. Altered fibrin clot properties in patients on long-term haemodialysis: relation to cardiovascular mortality. Nephrol Dial Transplant. 2008; 23: 2010-2015.

39 Morrissey JH, Choi SH, Smith SA. Polyphosphate: an ancient molecule that links platelets, coagulation, and inflammation. Blood. 2012; 119: 5972-5979.

40 Undas A, Szuldrzynski K, Stepien E, et al. Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: effects of inflammation and oxidative stress. Atherosclerosis. 2007; 196: 551-558.

41 Konieczynska M, Fil K, Bazanek M, et al. Prolonged duration of type 2 diabetes is associated with increased thrombin generation, prothrombotic fibrin clot phenotype and impaired fibrinolysis. Thromb Haemost. 2014; 111: 685-693.

42 Konieczyńska M, Bryk AH, Malinowski KP, et al. Interplay between elevated cellular fibronectin and plasma fibrin clot properties in type 2 diabetes. Thromb Haemost. 2017; 117: 1671-1678.

43 Undas A, Wiek I, Stepien E, et al. Hyperglycemia is associated with enhanced thrombin formation, platelet activation and fibrin clot resistance to lysis in patients with acute coronary syndrome. Diabetes Care. 2008; 31: 1590-1595.

44 Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev. 2007; 21: 131-142.

45 Marder VJ, Chute DJ, Starkman S, et al. Analysis of thrombi retrieved from cerebral arteries of patients with acute ischaemic stroke. Stroke. 2006; 37: 2086-2093.

46 Liebeskind DS, Sanossian N, Yong WH, et al. CT and MRI early vessel signs reflect clot composition in acute stroke. Stroke. 2011; 42: 1237-1243.

47 Undas A, Slowik A, Wolkow P, et al. Fibrin clot properties in acute ischaemic stroke: relation to neurological deficit. Thromb Res. 2010; 125: 357-361.

48 Carter AM, Kirby D, Englyst NA, et al. Fibrin structure/function in relation to stroke sub-types and post-stroke mortality. J Thromb Haemost. 2009; 6 (Suppl): OC-WE-114 [Abstract].

49 Bembenek JP, Niewada M, Siudut J, et al. Fibrin clot characteristics in acute ischaemic stroke patients treated with thrombolysis: the impact on clinical outcome. Thromb Haemost. 2017; 117: 1440-1447.

50 Goldman S, Prior SM, Bembenek JP, et al. Activation of blood coagulation and thrombin generation in acute ischemic stroke treated with rtPA. J Thromb Thrombolysis. 2017; 44: 362-370.

51 Undas A, Podolec P, Zawilska K, et al. Altered fibrin clot structure/function as a novel risk factor for cryptogenic ischaemic stroke. Stroke. 2009; 40: 1499-1501.

52 Matusik PT, Matusik PS, Kornacewicz-Jach Z, et al. Elevated NT-proBNP is associated with unfavorably altered plasma fibrin clot properties in atrial fibrillation. Int J Cardiol. 2017; 243: 244-250.

53 Rooth E, Wallen NH, Blombäck M, et al. Decreased fibrin clot network permeability and impaired fibrinolysis in the acute and convalescent phase of ischaemic stroke. Thromb Res. 2011; 127: 51-56.

54 Drabik L, Wołkow P, Undas A. Denser plasma clot formation and impaired fibrinolysis in paroxysmal and persistent atrial fibrillation while on sinus rhythm: association with thrombin generation, endothelial injury and platelet activation. Thromb Res. 2015; 136: 408-414.

55 Drabik L, Wołkow P, Undas A. Fibrin clot permeability as a predictor of stroke and bleeding in anticoagulated patients with atrial fibrillation. Stroke. 2017; 48: 2716-2722.

56 Meus R, Son M, Sobczyk D, et al. Prothrombotic state in patients with a left atrial appendage thrombus of unknown origin and cerebrovascular events. Stroke. 2016; 47: 1872-1878.

57 Celińska-Lowenhoff M, Iwaniec T, Padjas A, et al. Altered fibrin clot structure/function in patients with antiphospholipid syndrome: association with thrombotic manifestation. Thromb Haemost. 2014; 112: 287-296.

58 Mazur P, Sobczynski R, Zabczyk M, et al. Architecture of fibrin network inside thrombotic material obtained from the right atrium and pulmonary arteries: flow and location matter. J Thromb Thrombolysis. 2013; 35: 127-129.

59 Undas A, Stepien E, Rudzinski P, et al. Architecture of a pulmonary thrombus removed during embolectomy in a patient with acute pulmonary embolism. J Thorac Cardiovasc Surg. 2010; 140: e40-e41.

60 Martinez MR, Cuker A, Mills AM, et al. Enhanced lysis and accelerated establishment of viscoelastic properties of fibrin clots are associated with pulmonary embolism. Am J Physiol Lung Cell Mol Physiol. 2014; 306: L397-L404.

61 Undas A, Zawilska K, Ciesla-Dul M, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. Blood. 2009; 114: 4272-4278.

62 Xu S, Xu Z, Kim OV, et al. Model predictions of deformation, embolization and permeability of partially obstructive blood clots under variable shear flow. J R Soc Interface. 2017; 14: 20170441.

63 Pan X, Gong YY, Martinelli I, et al. Fibrin clot structure is affected by levels of particulate air 64 exposure in patients with venous thrombosis. Environ Int. 2016; 92-93: 70-76.

64 Siudut J, Świat M, Undas A. Altered Fibrin Clot Properties in Patients With Cerebral Venous Sinus Thrombosis: Association With the Risk of Recurrence. Stroke. 2015; 46: 2665-2668.

65 Zabczyk M, Plens K, Wojtowicz W, et al. Prothrombotic fibrin clot phenotype is associated with recurrent pulmonary embolism after discontinuation of anticoagulant therapy. Arterioscler Thromb Vasc Biol. 2017; 37: 365-373.

66 Siudut J, Grela M, Wypasek E, et al. Reduced plasma fibrin clot permeability and susceptibility to lysis are associated with increased risk of post-thrombotic syndrome. J Thromb Haemost. 2016; 14: 784-793.

67 Cieslik J, Mrozinska S, Broniatowska E, et al. Altered plasma clot properties increase the risk of recurrent deep vein thrombosis: a cohort study. Blood. In press.

68 Undas A, Cieśla-Dul M, Drążkiewicz T, et al. Altered fibrin clot properties are associated with residual vein obstruction: effects of lipoprotein(a) and apolipoprotein(a) isoform. Thromb Res. 2012; 130: e184-187.

69 Morris TA, Marsh JJ, Chiles PG, et al. Fibrin derived from patients with chronic thromboembolic pulmonary hypertension is resistant to lysis. Am J Respir Crit Care Med. 2006; 173: 1270-1275.

70 Morris TA, Marsh JJ, Chiles PG, et al. High prevalence of dysfibrinogenemia among patients with chronic thromboembolic pulmonary hypertension. Blood. 2009; 114: 1929-1936.

71 Marsh JJ, Chiles PG, Liang NC, et al. Chronic thromboembolic pulmonary hypertension-associated dysfibrinogenemias exhibit disorganized fibrin structure. Thromb Res. 2013; 132: 729-734.

72 Kopec G, Stępniewski J, Waligora M, et al. Staged treatment of central and peripheral lesions in chronic thromboembolic pulmonary hypertension. Pol Arch Med Wewn. 2016; 126: 97-99.

73 Janion-Sadowska A, Natorska J, Siudut J, et al. Plasma fibrin clot properties in the G20210A prothrombin mutation carriers following venous thromboembolism: the effect of rivaroxaban. Thromb Haemost. 2017; 117: 1739-1749.

74 Krzek M, Ciesla-Dul M, Zabczyk M, et al. Fibrin clot properties in women heterozygous for factor V Leiden mutation: effects of oral contraceptives. Thromb Res. 2012; 130: e216-e221.

75 Brzezińska-Kolarz B, Kolarz M, Wałach A, et al. Weight reduction is associated with increased plasma fibrin clot lysis. Clin Appl Thromb Hemost. 2014; 20: 832-837.

76 Ajjan RA, Standeven KF, Khanbhai M, et al. Effects of aspirin on clot structure and fibrinolysis using a novel in vitro cellular system. Arterioscler Thromb Vasc Biol. 2009; 29: 712-717.

77 Svensson J, Bergman AC, Adamson U, et al. Acetylation and glycation of fibrinogen in vitro occur at specific lysine residues in a concentration dependent manner: a mass spectrometric and isotope labeling study. Biochem Biophys Res Commun. 2012; 421: 335-342.

78 Antovic A, Perneby C, Ekman GJ, et al. Marked increase of fibrin gel permeability with very low dose ASA treatment. Thromb Res. 2005; 116: 509-517.

79 Fatah K, Beving H, Albage A, et al. Acetylsalicylic acid may protect the patient by increasing fibrin gel porosity. Is withdrawing of treatment harmful to the patient? Eur Heart J. 1996; 17: 1362-1366.

80 Undas A, Celinska-Lowenhoff M, Lowenhoff T, et al. Statins, fenofibrate, and quinapril increase clot permeability and enhance fibrinolysis in patients with coronary artery disease. J Thromb Haemost. 2006; 4: 1029-1036.

81 Ząbczyk M, Majewski J, Karkowski G, et al. Vitamin K antagonists favourably modulate fibrin clot properties in patients with atrial fibrillation as early as after 3 days of treatment: Relation to coagulation factors and thrombin generation. Thromb Res. 2015; 136: 832-838.

82 He S, Blombäck M, Bark N, et al. The direct thrombin inhibitors (argatroban, bivalirudin and lepirudin) and the indirect Xa-inhibitor (danaparoid) increase fibrin network porosity and thus facilitate fibrinolysis. Thromb Haemost. 2010; 103: 1076-1084.

83 Blombäck M, He S, Bark N, et al. Effects on fibrin network porosity of anticoagulants with different modes of action and reversal by activated coagulation factor concentrate. Br J Haematol. 2011; 152: 758-765.

84 Zolcinski M, Ciesla-Dul M, Undas A. Effects of atorvastatin on plasma fibrin clot properties in apparently healthy individuals and patients with previous venous thromboembolism. Thromb Haemost. 2012; 107: 1180-1182.

85 Undas A, Brummel-Ziedins KE, Mann KG. Anticoagulant effects of statins and their clinical implications. Thromb Haemost. 2014; 111: 392-400.

86 Undas A, Topór-Madry R, Tracz W. Simvastatin increases clot permeability and susceptibility to lysis in patients with LDL cholesterol below 3.4 mmol/l. Pol Arch Med Wewn. 2009; 119: 354-359.

87 Undas A, Kaczmarek P, Sladek K, et al. Fibrin clot properties are altered in patients with chronic obstructive pulmonary disease. Beneficial effects of simvastatin treatment. Thromb Haemost. 2009; 102: 1176-1182.